



ELSEVIER

Available online at www.sciencedirect.com ScienceDirect

Experimental and Toxicologic Pathology ■ (■■■■) ■■■-■■■

**EXPERIMENTAL
AND
TOXICOLOGIC
PATHOLOGY**www.elsevier.de/etp

Antioxidant effects of methionine, α -lipoic acid, *N*-acetylcysteine and homocysteine on lead-induced oxidative stress to erythrocytes in rats

Emrah Caylak^a, Metin Aytekin^{b,*}, Ihsan Halifeoglu^a^aDepartment of Biochemistry and Clinical Biochemistry, Firat University, Medical School, Elazig, Turkey^bDepartments of Pathobiology, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA

Received 4 October 2007; accepted 20 November 2007

Abstract

Lead, widely used in industry, is a great environmental health problem. Many studies have examined its effects on the health of both humans and animals. Experimental studies have shown that sulphur-containing antioxidants have beneficial effects against the detrimental properties of lead. The present study was designed to investigate markers of oxidative stress (hemoglobin (Hb) in whole blood, malondialdehyde (MDA) in sera; superoxidase dismutase (SOD) and glutathione peroxidase (GSH-Px) in erythrocyte hemolysate and vitamins A and E in plasma) in rats given lead (2000 ppm) with or without sulphur-containing antioxidants (L-methionine (Met) (100 mg/kg/day), *N*-acetylcysteine (NAC) (800 mg/kg/day), L-homocysteine (Hcy) (25 mg/kg/day), lipoic acid (LA) (50 mg/kg/day)) in their water for 5 weeks. In the lead group, Hb and plasma vitamin E levels were significantly lower whereas MDA levels were significantly higher compared to controls ($p < 0.05$). Hb levels in lead-methionine and lead-LA groups were significantly higher than the lead group ($p < 0.01$). MDA levels were reduced in all groups compared to the lead group ($p < 0.01$). There was a decrease below control values in erythrocyte SOD ($p < 0.01$) and GSH-Px ($p < 0.05$) levels in the lead-LA group. Plasma vitamin A levels were significantly high in lead-methionine group compared to lead group ($p < 0.01$). In conclusion, the data suggests that oxidative stress induced by lead is reduced by sulphur-containing compounds.

© 2008 Elsevier GmbH. All rights reserved.

Keywords: Lead; Oxidative stress; Methionine; Lipoic acid; *N*-Acetylcysteine; Homocysteine

Introduction

Lead, commonly used in industrialized countries, adversely affects human and animal physiological, biochemical, and behavioral functions. The mechanism of lead toxicity may be due, in part, to disruption of the prooxidant/antioxidant balance, leading to tissue injury

via oxidative damage to critical biomolecules such as lipids, proteins, and DNA. The hematological system is the major target of low level lead exposure. Previous studies have suggested that lead-induced oxidative damage in red blood cells may result from direct interaction of lead with their membranes, inducing lipid peroxidation (Hermes-Lima et al., 1991; Sandhir et al., 1994) and inhibiting heme and hemoglobin synthesis (Warren et al., 1998).

The strong scientific interest in the role of antioxidants has expanded the focus of research from reducing

*Corresponding author. Tel.: +1 216 445 6542;

fax: +1 216 636 0104.

E-mail address: aytekim@ccf.org (M. Aytekin).

the oxidative stress of lead exposure to improving the prooxidant/antioxidant balance of cells. Thiol-containing compounds bind lead at their –SH (thio) groups and have antioxidant features. Therefore, thiol-containing antioxidants may be useful as a component of an effective treatment for lead poisoning.

Methionine acts a precursor amino acid for glutathione which protects the cells from oxidative damage and plays vital role in detoxification (Reed and Orrenius, 1977; Reed, 1990). In addition, methionine has been shown to chelate lead and remove it from tissues (Patra et al., 2001). α -Lipoic acid (LA), is a co-enzyme of pyruvate and the α -ketoglutarate dehydrogenase multienzyme complex of the tricarboxylic acid cycle (Patel and Roche, 1990), and has metal chelating, free radical scavenging, and antioxidant-regenerating abilities (Packer et al., 1995). *N*-Acetylcysteine (NAC) has antioxidant capacity to lead, including oxidative stress via stimulating glutathione synthesis, thereby maintaining intracellular glutathione levels and scavenging reactive oxygen species (Ercal et al., 1996). In addition, NAC also has some chelating action on lead (Aruoma et al., 1989). Homocysteine (Hcy), a thiol formed by demethylation of methionine, is at moderately high levels, a known independent risk factor for atherosclerosis and increased vascular dysfunction (Refsum and Ueland, 1998). However, according to some authors, Hcy, contains a thiol group, displays an antioxidant effect on cellular systems at micromolar levels (Zappacosta et al., 2000).

In the present study, we investigated the beneficial effects of thiol-containing antioxidants on altered oxidative stress parameters and antioxidant enzyme levels with lead treatment. We determined malondialdehyde (MDA) levels as an indicator of lipid peroxidation. Oxidative stress status was described by determination of hemoglobin (Hb), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and vitamins A and E levels. We also investigated whether Hcy has an antioxidant effect in response to lead exposure.

Methods

Chemicals

Lead acetate and NAC were purchased from Merck (Darmstadt, Germany). All other chemicals were purchased from Sigma (St. Louis, MO, USA). HPLC grade reagents were used in vitamins A and E analysis.

Animals

Wistar-albino male rats were procured from Firat University Experimental Research Unit Animals

weighed 150–200 g. Animals were utilized as per the permission from the Medical Faculty Animal Ethics Committee. They were fed a standard rat pellet diet and had free access to water. The rats were housed in stainless steel cages in a temperature-controlled room (20–22 °C) with a 12 h light and 12 h dark exposure.

Experimental design

The animals were randomized into six groups of 10 animals each. All groups were given only standard rat feed and water during the 1st week. After this period of adjustment to their environment, group I ($n = 10$) served as the control and was given only standard rat chow and water for 5 weeks. Group II ($n = 10$) received 2000 ppm lead acetate in their drinking water for 5 weeks. Group III ($n = 10$) received 2000 ppm lead acetate in their drinking water for 5 weeks and 100 mg/kg/day methionine dissolved in water and administered in their drinking water. Group IV ($n = 10$) was treated like group III, except that it received 25 mg/kg/day i.p. LA dissolved in a 1:1 ratio with ethyl alcohol and administered intraperitoneally for 5 weeks. At the end of the study, two rats had died from peritonitis. Group V ($n = 10$) received water containing 2000 ppm lead acetate and 800 mg/kg/day NAC. Group VI ($n = 10$) received 2000 ppm lead acetate and 50 mg/kg/day Hcy in their water. The rats were housed in separate cages to ensure the correct dose was received. At the end of the 5th week, the animals were sacrificed by cervical decapitation. The blood samples were collected in lead free tubes using heparin and EDTA as anticoagulant or tubes without anticoagulants. Plasma and serum were removed by centrifugation for 10 min at 3000 rpm. The red blood cells were washed three times with an equal volume of cold saline. The samples were maintained at –20 °C before performing assays (not longer than 7 days).

Assays

Hb determination

Hb concentrations in whole blood were spectrophotometrically analyzed by the cyanomethemoglobin method (Leong et al., 2003). Blood samples (20 μ l) were mixed with 5 ml Drabkin's solution (0.1% sodium bicarbonate, 0.005% potassium cyanide, and 0.02% potassium ferricyanide) for hemoglobin determination. Hb standard was purchased from Sigma (St. Louis, MO, USA).

MDA determination

MDA concentrations were measured as TBARS (thiobarbituric acid reactive substances) according to a modified version of Satoh's (1978) and Yagi's (1984)

methods. In brief, 0.3 ml serum was mixed with 2.4 ml 1/12 N H₂SO₄ in a centrifuge tube and shaken gently. After, 0.3 ml 10% (v/v) phosphotungstic acid was added to the tube and it was left to stand at room temperature for 5 min; the mixture was then centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the sediment was mixed with 1.5 ml water. The centrifugation was repeated and the supernatant was discarded again. The sediment was resuspended in 4.0 ml water and 1.0 ml fresh thiobarbituric acid (TBA) reagent (1:1, v/v 0.67% TBA and glacial acetic acid), mixed thoroughly and heated in a bath of boiling water for 1 h. After cooling in cold water, the resulting chromogen was extracted with 3.0 ml *n*-butyl alcohol by vigorous shaking. The organic phase was separated by centrifugation at 3000 rpm for 10 min, and its absorbency was recorded at a wavelength of 530 nm. The level of absorbency was converted into nmol/ml MDA from a standard curve generated with 1.1.3.3-tetraethoxypropane (SIGMA).

SOD and GSH-Px activities determination

Cu/Zn SOD activity in erythrocytes was measured by the rate of inhibition of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) reduction (Ransod SD 125, Randox Lab.) and expressed in U/g of hemoglobin (Hb). GSH-Px activity in whole blood was measured by a modification of the method of Paglia and Valentine (1967). GSH-Px catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured (Ransel RS 505, Randox Lab.) in an Olympus AU 600 analyzer at 37 °C. An internal control system based on commercial lyophilized blood (Ransod control SD 126 and Ransel control SC 692, Randox Lab.) was used for validation of analytical runs. The activities of SOD and GSH-Px were expressed in U/g of Hb.

Determination of vitamins A and E by HPLC

Plasma vitamins A and E levels were estimated by HPLC using Recipe commercial kits (Recipe Chemical and Instruments GmbH, Munich, Germany). For HPLC adjustment, the detector was selected as UV, total analysis time set to 8 min, and a flow-rate was adjusted to 1.5 ml/min and injection volume set to 20 µl. Special commercial vitamin-kit columns were used at 30 °C. Vitamin A is monitored at 325 nm, while vitamin E is monitored at 295 nm. Briefly, 100 µl of precipitant P, solution came with the kit which contains 3 µg internal standard, was added to 100 µl plasma. After vortexing for 30 s, the samples were centrifuged and thereafter 20 µl supernatant injected into the HPLC-

system (Shimadzu 10A VP, Japan). Coefficient of variability over time using control plasma was less than 7% for vitamin A and 5% for vitamin E. The recovery rate was above 90–100% for all parameters.

Statistics

The results are expressed as mean ± S.D. Analysis of the data was performed by one-way analysis of variance (ANOVA) and subsequent analysis was performed using the Tukey test. The *p* values smaller than 0.05 were selected to indicate statistical significance between groups.

Results

Hb concentrations

The table displays the results of RBC Hb measurements in control, lead-exposed group and all lead-antioxidant groups. Animals given lead for 5 weeks had significantly lower Hb concentrations when compared to control rats (*p* < 0.01). The Hb levels of NAC and Hcy treated, lead-exposed rats were significantly lower than controls (*p* < 0.01). Methionine and LA treatment slightly decreased Hb concentrations compared to controls, although not significantly (*p* > 0.05). Although NAC and Hcy supplements did not appear to change Hb concentrations (*p* > 0.05), methionine and LA administrations were found to be very effective in increasing Hb levels when compared to those in the lead-group (*p* < 0.01).

MDA concentrations

The table shows the results of serum MDA concentrations in all groups. The MDA level of lead-group (*p* < 0.01) and lead-LA group (*p* < 0.05) were significantly higher than the control values. Statistically significant decreases were observed in MDA concentrations in all lead-antioxidants groups when compared to lead-group (*p* < 0.01).

Activity of antioxidant enzymes

The table shows the activity of antioxidant enzymes SOD and GSH-Px in RBCs. Significant decreases in SOD activity of erythrocytes were recorded in lead-LA group as compared with controls (*p* < 0.05) and lead-group (*p* < 0.01). Lead, methionine and NAC supplementation increased the SOD activities although Hcy decreased SOD activities but these effects were not significantly different than the control. No significant change in SOD activity was recorded in any of the

Table 1. Hb and MDA concentrations; SOD and GSH activities and, vitamins A and E status

Parameter	Group					
	Control (n:10)	Pb (n:10)	Pb + Met (n:10)	Pb + LA (n:8)	Pb + NAC (n:10)	Pb + Hcy (n:10)
Hb (g/dl)	16.52±1.72	12.71±1.16 ^a	15.65±2.54 ^c	16.51±1.22 ^c	13.58±1.55 ^a	13.15±1.64 ^a
MDA (nmol/ml)	0.35±0.06	0.66±0.06 ^a	0.29±0.03 ^c	0.45±0.10 ^{b,c}	0.40±0.09 ^c	0.28±0.07 ^c
SOD (U/gHb)	1954±799	2477±574	2257±418	1104±363 ^{b,c}	2345±608	1872±290
GSH-Px (U/gHb)	28.8±8.7	30.0±4.6	29.5±5.6	21.6±3.5 ^b	29.3±3.9	29.7±2.0
Vit A (mg/L)	1.5±0.2	1.2±0.3	1.8±0.5 ^b	1.1±0.1 ^c	1.4±0.2	1.1±0.3 ^c
Vit E (mg/L)	16.3±1.9	12.2±2.6 ^a	13.6±4.4	10.1±1.5 ^a	14.2±1.5	8.5±0.9 ^{a,d}

All values represent mean±S.D. for 8–10 samples.

^a $p < 0.01$ vs. control;

^b $p < 0.05$ vs. control;

^c $p < 0.01$ vs. lead group;

^d $p < 0.05$ vs. lead group.

lead–antioxidant groups except for the lead–LA group compared to the lead group.

Each preventive regiment combined with and lead treatment could not significantly change the values to restore normalcy. Supplementing lead with LA lowered the GSH-Px activities in RBCs as compared with lead-group ($p < 0.05$), while the other antioxidants did not.

Antioxidant vitamin status

The plasma antioxidant vitamins A and E levels are given in Table 1. The plasma vitamin A levels in rats on the lead–LA group and lead–Hcy groups were significantly lower ($p < 0.01$) than in controls, although no significant decrease was observed in the lead-group and lead–NAC group. A significant increase could be noted in vitamin A levels in plasma from the methionine supplemented group compared to lead group ($p < 0.05$).

Plasma vitamin E levels were significantly lower in rats given lead alone or lead in conjunction with LA or Hcy ($p < 0.01$), while the other antioxidant supplemented groups showed no significant differences.

Discussion

Lead, a common environmental occupational toxic heavy metal, is known to have indirect oxidative effects on biological systems and cells. Lead exposure induces severe oxidative damage in RBCs by inhibiting heme and hemoglobin synthesis and changing erythrocyte morphology and survival (Leggett, 1993).

Oxidative stress also leads to lipid peroxidation in RBC membranes, autooxidation of hemoglobin, and

limited repair processes, leading to decreased survival (Rice-Evans and Baysal, 1987).

The present study was designed to investigate oxidative stress parameters (Hb in MDA in serum, SOD and GSH-Px activities in erythrocyte hemolysate, and vitamins A and E in plasma) in rats administered lead in conjunction with sulphur-containing antioxidants.

One of the clinical symptoms of lead intoxication is anemia, due to both impaired hemoglobin synthesis and damaged erythrocyte membranes (Waldron, 1966; Sugawara et al., 1991). The results of our study demonstrated that administration of lead acetate for 5 weeks induced a toxic effect on RBCs as indicated by a significant reduction in Hb concentrations.

In vivo, lead-induced lipid peroxidation results in the formation of aldehydic by-products such as MDA. In the present work, the increased MDA levels in serum of lead-exposed rats were statistically significant confirming previous studies (Ercal et al., 1996; Gurer et al., 1998).

In lead intoxication, lead induces generation of reactive oxygen species such as hydrogen peroxide (H_2O_2), superoxide ion ($O_2^{\cdot-}$), singlet oxygen and hydroxyl radical (HO^{\cdot}). SOD and GSH-Px play an important role in protecting the cells against the toxic effects of $O_2^{\cdot-}$ and peroxides, H_2O_2 , respectively, and may be upregulated in response to lead exposure.

In this study, a group of rats exposed to lead had slightly higher activities of RBC SOD and GSH-Px compared to controls. Experimental and clinical research on lead-exposed animals and workers has noted different effect of lead on the activities of SOD and GSH-Px. Some studies show a drop of SOD and GSH-Px activities in lead exposed rats (Sivaprasad et al., 2004), while other studies found higher activity of SOD (Soltaninejad et al., 2003). In our research, no significant

effects of exposure to lead on the activities of SOD and GSH-Px were indicated. We also examined the non-enzymatic antioxidant system status, e.g. vitamins A and E in plasma of rats exposed to lead. In lead administered rats, there is a decrease in vitamin levels, similar to other reports (Attri et al., 2003).

Lead is known to inhibit several antioxidant enzymes having functional –SH groups (Vallee and Ulmer, 1972). In blood, 70% of lead is bound to delta-aminolevulinic acid dehydratase (ALAD), which is inhibited by lead via direct binding of lead to the eight SH groups that are essential for the catalytic activity of the enzyme (Fujita et al., 1982; Bernard and Lauwerys, 1987). Thirty percent of lead is connected to other enzymes containing SH groups; these enzymes are also inhibited by lead. Glucose 6-phosphate dehydrogenase (G6PD) has many SH groups and binds lead (Goyer and Rhyne, 1973). In addition, it is found that ALAD enzyme concentrations are elevated in lead-exposed rats and humans to compensate for inhibition by lead (Fujita et al., 2002). This may be caused by increased synthesis of this enzyme in the erythroblastic system of bone marrow, 5–7 days after lead exposure increased ALAD concentrations can be determined in peripheral RBCs (Fujita et al., 1981). Furthermore, Fujita and Ishihara (1988) showed that in a cell-free analysis, *de novo* synthesis of ALAD was induced by lead. Probably, SOD and GSH-Px levels of RBCs in peripheral or bone marrow also increase to compensate lead induced enzyme inhibition.

On the other hand, the time exposure of lead in the people protractedly exposed to lead compounds is a few or several years (Solliway et al., 1996; Machartova et al., 2000). Most research on lead toxicity in animals utilizes a 4–5 week lead exposure period followed by a 6th week in which they receive only water or therapeutic antioxidant compounds (Gurer et al., 1998; Othman and El Missiry, 1998). Unlike the previous studies, in our study, lead and preventative antioxidant compounds were administered simultaneously in water to rats during the 5 weeks.

In conclusion, the hematological system is a major target of low-level lead exposure. The results of the present study suggest that treatment with sulfur-containing antioxidant molecules has a potentially protective role in the scavenging of free radicals generated indirectly by lead, including those formed during the xenobiotic-induced lipid peroxidation and those caused by the decreased antioxidant enzyme activities and vitamin levels. Therefore, additional dietary supplementation with sulfur-containing antioxidants may be beneficial to workers in paint, glass, and other industries in which lead exposure is common. However, further studies are required to verify the protective effects of these antioxidants, particularly at co-exposure to different combinations and doses in the presence of oxidative damage due to chronic low dose exposure to lead in animals and humans.

Acknowledgments

The authors are thankful to assistants of Firat University Medical Scholl Biochemical Laboratory for determining tissue total antioxidant capacities. Dr. Emrah Caylak was supported by 801 from the Projects of Firat University Academical Research. The authors also gratefully acknowledge Allison Janocha.

References

- Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med* 1989;6:593–7.
- Attri J, Dhawan V, Mahmood S, Pandhi P, Parwana HK, Nath R. Effect of vitamin C supplementation on oxidative DNA damage in an experimental model of lead-induced hypertension. *Ann Nutr Metab* 2003;47:294–301.
- Bernard A, Lauwerys R. Metal-induced alterations of delta-aminolevulinic acid dehydratase. *Ann NY Acad Sci* 1987; 514:41–7.
- Ercal N, Treeratphan P, Lutz P, Hammond TC, Matthews RH. *N*-acetylcysteine protects Chinese hamster ovary (CHO) cells from lead-induced oxidative stress. *Toxicology* 1996;108:57–64.
- Fujita H, Ishihara N. Evidence of the induction of *de novo* synthesis of delta-aminolaevulinic acid dehydratase by lead. *Br J Ind Med* 1988;45:710–2.
- Fujita H, Orii Y, Sano S. Evidence of increased synthesis of delta-aminolevulinic acid dehydratase in experimental lead-poisoned rats. *Biochim Biophys Acta* 1981;678:39–50.
- Fujita H, Sato K, Sano S. Increase in the amount of erythrocyte delta-aminolevulinic acid dehydratase in workers with moderate lead exposure. *Int Arch Occup Environ Health* 1982;50:287–97.
- Fujita H, Nishitani C, Ogawa K. Lead, chemical porphyria, and heme as a biological mediator. *Tohoku J Exp Med* 2002;196:53–64.
- Goyer RA, Rhyne BC. Pathological effects of lead. *Int Rev Exp Pathol* 1973;12:1–77.
- Gurer H, Ozgunes H, Neal R, Spitz DR, Ercal N. Antioxidant effects of *N*-acetylcysteine and succimer in red blood cells from lead-exposed rats. *Toxicology* 1998;128:181–9.
- Hermes-Lima M, Pereira B, Bechara EJ. Are free radicals involved in lead poisoning? *Xenobiotica* 1991;21:1085–90.
- Leggett RW. An age-specific kinetic model of lead metabolism in humans. *Environ Health Perspect* 1993;101:598–616.
- Leong WI, Bowlus CL, Tallkvist J, Lonnerdal B. DMT1 and FPN1 expression during infancy: developmental regulation of iron absorption. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G1153–61.
- Machartova V, Racek J, Kohout J, Senft V, Trefil L. [Effect of antioxidant therapy on indicators of free radical activity in workers at risk of lead exposure]. *Vnitr Lek* 2000;46:444–6.
- Othman AI, El Missiry MA. Role of selenium against lead toxicity in male rats. *J Biochem Mol Toxicol* 1998;12: 345–9.

- Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radic Biol Med* 1995;19:227–50.
- Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158–69.
- Patel MS, Roche TE. Molecular biology and biochemistry of pyruvate dehydrogenase complexes. *FASEB J* 1990;4:3224–33.
- Patra RC, Swarup D, Dwivedi SK. Antioxidant effects of alpha tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. *Toxicology* 2001;162:81–8.
- Reed DJ. Glutathione: toxicological implications. *Annu Rev Pharmacol Toxicol* 1990;30:603–31.
- Reed DJ, Orrenius S. The role of methionine in glutathione biosynthesis by isolated hepatocytes. *Biochem Biophys Res Commun* 1977;77:1257–64.
- Refsum H, Ueland PM. Recent data are not in conflict with homocysteine as a cardiovascular risk factor. *Curr Opin Lipidol* 1998;9:533–9.
- Rice-Evans C, Baysal E. Iron-mediated oxidative stress in erythrocytes. *Biochem J* 1987;244:191–6.
- Sandhir R, Julka D, Gill KD. Lipoperoxidative damage on lead exposure in rat brain and its implications on membrane bound enzymes. *Pharmacol Toxicol* 1994;74:66–71.
- Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 1978;90:37–43.
- Sivaprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. *J Nutr Biochem* 2004;15:18–23.
- Solliway BM, Schaffer A, Pratt H, Yannai S. Effects of exposure to lead on selected biochemical and haematological variables. *Pharmacol Toxicol* 1996;78:18–22.
- Soltaninejad K, Kebriaeezadeh A, Minaiee B, Ostad SN, Hosseini R, Azizi E, et al. Biochemical and ultrastructural evidences for toxicity of lead through free radicals in rat brain. *Hum Exp Toxicol* 2003;22:417–23.
- Sugawara E, Nakamura K, Miyake T, Fukumura A, Seki Y. Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. *Br J Ind Med* 1991;48:239–42.
- Vallee BL, Ulmer DD. Biochemical effects of mercury, cadmium, and lead. *Annu Rev Biochem* 1972;41:91–128.
- Waldron HA. The anaemia of lead poisoning: a review. *Br J Ind Med* 1966;23:83–100.
- Warren MJ, Cooper JB, Wood SP, Shoolingin-Jordan PM. Lead poisoning, haem synthesis and 5-aminolaevulinic acid dehydratase. *Trends Biochem Sci* 1998;23:217–21.
- Yagi K. Assay for blood plasma or serum. *Methods Enzymol* 1984;105:328–31.
- Zappacosta B, Mordente A, Persichilli S, Giardina B, De Sole P. Effect of homocysteine on polymorphonuclear leukocyte activity and luminol-dependent chemiluminescence. *Luminescence* 2000;15:257–60.